THE MECHANICS OF COPULATION IN AEDES AEGYPTI[1]

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Although the sclerites associated with the genitalia of mosquitoes have frequently been illustrated and form the base for the classification of these insects, their functions are incompletely understood. Several published reports describe the act of copulation in mosquitoes, but none record the precise manner in which the aedeagus is mobilized. Russell and Mohan (1939) fixed copulating pairs of *Anopheles stephensi* and observed that the male claspers engaged the base of one of the terminal abdominal segments of the female rather than the cerci, while the opposing action of the claspettes secured the female ventrally. In contrast, *Deinocerites* males employed their enormously elongated ninth tergite lobes for holding the female (Komp, 1956) and apparently did not use their relatively small claspers. Neither study recorded observations of the means of sperm induction. Rees and Onishi (1951) stated that the aedeagus of *Culiseta inornata* moved "in and out" in a relatively straight line and indicated that it was thereby directed deep into the common oviduct. However, they did not recognize the copulatory bursa of the culicine female (Brelje, 1924) and did not report direct observations of the aedeagus during copulation. Wheeler and Jones (1960) observed that the aedeagus "moves in a ventrally directed arc" in *A. aegypti* but did not report its actual position in coitus. The claspers are apparently the primary holdfast organs in both *A. aegypti* and *C. inornata*, the distal segment being depressed over the cerci of the female.

The present report describes the mechanism through which seminal material is transferred from the *A. aegypti* male to the female. The anatomy of the genital structures is re-described in light of these studies and the course of seminal fluid from the seminal vesicle of the male to the spermatheca of the female traced.

Materials and Methods

Specimens were derived from a Johns Hopkins University colony of *A. aegypti* that has been maintained in laboratory culture for more than 20 years. Larvae were reared, using conventional techniques, and pupae were separated as to sex on the basis of size. They were then isolated in individual test tubes where emergence occurred. Adults were subsequently held without food for three to four days.

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Pairs were permitted to copulate naturally by introducing virgin females into standard lantern chimneys containing 15 to 20 males. Their activity was observed and the duration of genital contact recorded. Alternatively, copulation of restrained pairs was induced using a modification of the McDaniel and Horsfall (1957) technique. Nitrogen was used to anesthetize males and ether to anesthetize females. Carbon dioxide anesthesia appeared to interfere with copulation.

Mosquitoes in various stages of copulation, as well as individual specimens, were killed by freezing. They were taken from the lantern chimneys by aspirator tube and quickly transferred to a freezing chamber immersed in an alcohol-solid CO_2 bath. Copulating pairs retained genital contact throughout this process. Subsequently, material was studied in one of several ways. While still frozen, they were fixed in Newcomer's solution (Newcomer, 1953) at solid CO_2 temperature. Some were transferred to 95% alcohol and then to beechwood creosote for dissection. Permanent mounts of 73 males and 36 females were prepared in this manner. Other specimens were imbedded after fixation, sectioned serially at 6–8 μ and stained with hematoxylin-azure II-eosin. Thirty-eight females and 15 males were studied in this manner. More than 20 additional specimens were prepared in the conventional manner by maceration in KOH and staining with acid-fuchsin. Supplemental observations were made with more than 100 males and females that were freshly dissected in *Drosophila* Ringer's solution.

Observations

Behavior patterns preceding copulation. Males attempted to attach themselves to flying females or to a tuning fork simulating the sound of the female's wings, as described by Roth (1948). When males established contact with a female whose wings were in motion, they tended to retain their hold even after wing motion had ceased. However, males did not retain contact with females whose wings were at rest when contact was established. Twenty 4-day-old virgin females, whose wings had been removed, were placed in a lantern chimney with 20 virgin males of the same age. None was inseminated after 24 hours. The experiment was repeated, but with one-half of the females wingless. Only the winged females (10 out of 10) were inseminated during the 24-hour exposure period. The initial contact was therefore established chiefly during flight, and this appeared to be the invariable rule when copulation was directly observed.

Genital contact, however, rarely occurred during flight in the small mating chambers. More than 200 copulating pairs were systematically observed and only in three were the abdomens in contact when they landed upon the container's floor. The remainder flew about momentarily after pairing and generally landed upon the floor with the male's tarsi in contact with the female's. Thereupon, the males made strenuous attempts to invert themselves beneath the females, if they were not already in this position, while arching their abdomens and moving their claspers in a grasping manner. The females' wings were not in motion while this occurred. Copulation was aborted about half the time as males attempted to invert themselves. Once tarsal contact was lost it was never regained while the pair was at rest. When a copulating pair was disturbed, the mosquitoes frequently flew off with their genitalia in contact and assumed the mating postures described by Roth (1948).

Whole-mount preparations of male terminalia.

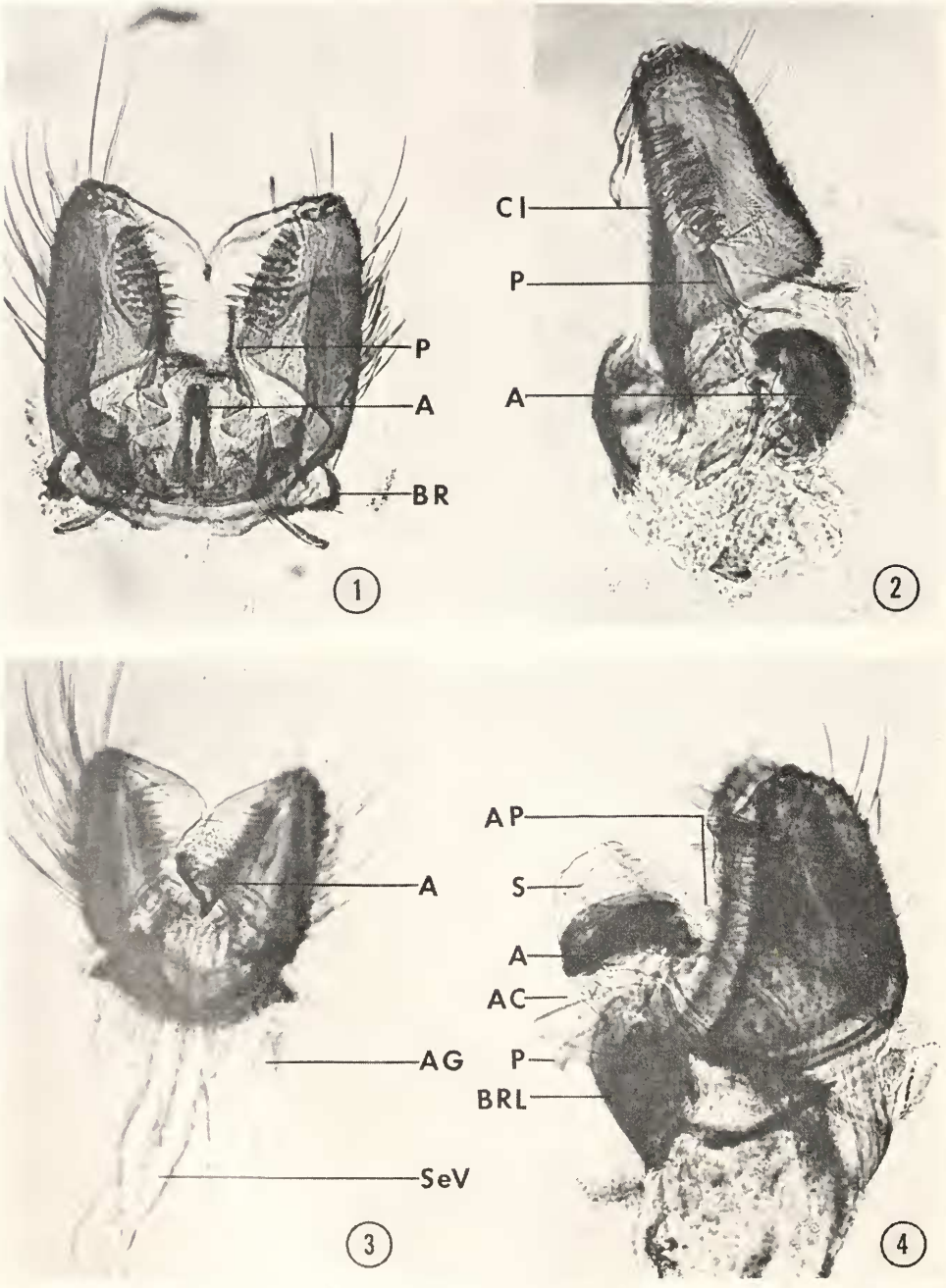


FIGURE 1. Ventral aspect of non-copulating male, showing relationship of paraprocts (P) to aedeagus (A) and position of basal ring (BR).

The cuticular sheet from which the clasper is derived is partially rolled into a cylinder. Its apodeme is continuous with the body of the clasper but is rotated so as to reverse its dorso-ventral orientation (illustrated by Christophers, 1960, but not by Hodapp and Jones, 1961). The dorsal root of the apodeme bears a thickened ridge upon the ventral surface of which the apodeme of the aedeagus articulates. The basal segment is inserted in the membranous area closing each clasper's cylindrical wall, and is continuous with the bridge connecting it with the basal segment in the opposite clasper. These segments are thickly studded with strong setae and each bears two or three prominent setae whose ends are finely tapered and bent. These setae, apparently specialized sensillae, are innervated from the last abdominal ganglion. The distal lobe is rod-like, and, at its end, bears a socketed claw.

The copulatory apparatus of the male is supported by the basal ring which is derived from the combined plates of the ninth abdominal segment. The dorsal portion of the ring appears to be sessile in relation to the preceding segment. The ventral portion, however, articulates with the dorsal at a point just distal to the base of the prominent ventral lobes. These large, cup-like lobes shield the anus on its ventral surface.

Two membranous structures arise from within the basal ring and envelop the aedeagus and paraprocts (Figs. 4, 7). Ventrally, the anal cone (proctiger) is continuous with the inner margin of the lobes of the basal ring and with the dorsal surface of the anus. This prominent, cone-shaped structure is continuous on its dorsal surface with the ventral margins of the aedeagus and of the claspers. In ventral view, the anal cone obscures the aedeagus and all but the hooked projections of the paraprocts. The lateral lobes of the paraprocts are inserted on its dorsal surface. The aedeagal pouch arises dorsally from the bridge that connects the clasper's basal lobes, and is continuous with the base of the aedeagus on its ventral margin. This membranous ridge shields the dorsal aspect of the aedeagus and paraprocts.

Seven paired muscles associated with the sclerotized portions of the copulatory apparatus are illustrated in Figure 8. They include: (1) a small muscle which extends the distal segment of the clasper; (2) a muscle that retracts the distal lobe of the clasper against the basal lobe; (3) a very heavy muscle which retracts the clasper apodeme against the body of the clasper; (4) a prominent group of muscle fibers which connects the clasper and the dorsal portion of the basal ring and serves to extend the entire copulatory apparatus dorsally; (5) a relatively small muscle that retracts the apodeme of the aedeagus against the distal portion of the apodeme of the clasper; (6) a long, slender muscle that retracts the lateral segment of the paraproct against the apodeme of the clasper; and (7) a muscle that draws the apodeme of the aedeagus against the body of the clasper.

FIGURE 2. Lateral aspect.

FIGURE 3. Ventral aspect of copulating male showing everted aedeagus and positions of seminal vesicles (SeV) and accessory glands (AG). Note constriction in accessory gland of this ejaculating male and presence of ejected seminal material.

FIGURE 4. Lateral view showing ejected seminal material (S), position of paraprocts during copulation and position of anal cone (AC) and everted aedeagal pouch (AP).

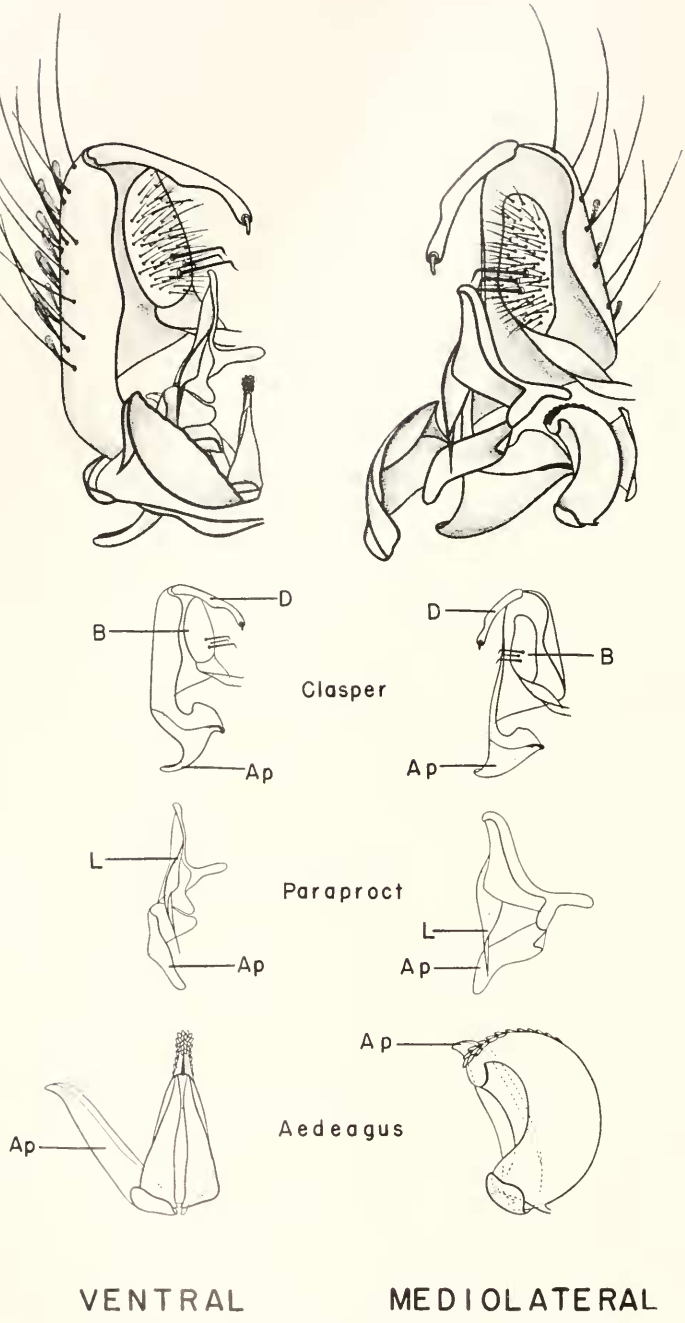


FIGURE 5. Terminalic sclerites of non-copulating male, hemisected and with functional components separated. (Corresponds to Figures 1, 2.)

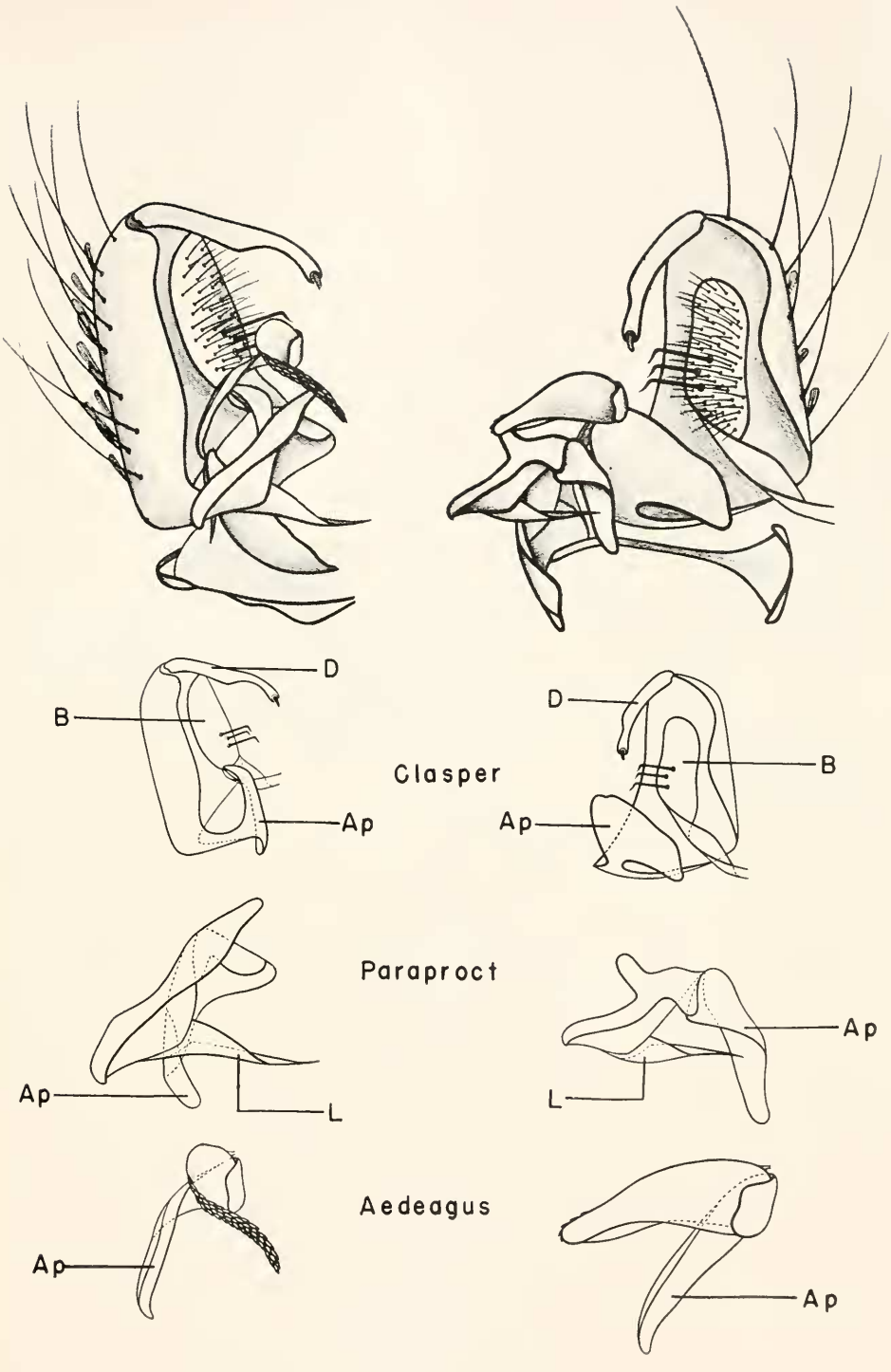


FIGURE 6. Terminalic sclerites of copulating male, hemisected and with functional components separated. (Corresponds to Figures 3, 4.)

The gross morphology of the internal copulatory structures of the male has been described by Christophers (1960), Hodapp and Jones (1961) and by Lum (1961). The following observations amplify these descriptions. The ejaculatory duct leads directly into the genital pore which is located near the base of the aedeagus (Fig. 7). The duct wall is composed of three layers of tissue: an innermost layer of thin cuticle, a low epithelium, and a thin overlying syncytium of transverse muscle fibers. When at rest the duct is contracted and virtually closed, and no internal movements were noted when it was dissected free of surrounding

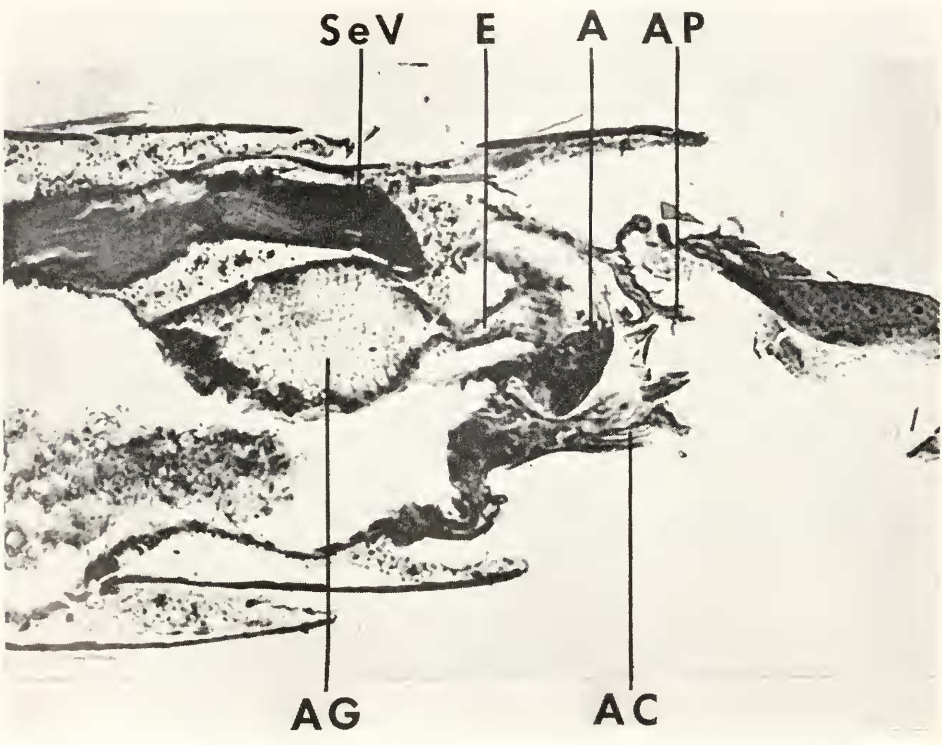


FIGURE 7. Sagittal section of non-copulating male, showing insertion of ejaculatory duct (E) in the aedeagus and placement of aedeagus between aedeagal pouch (AP) and anal cone (AC).

tissue. The paired accessory glands arise near the anterior end of the ejaculatory duct. The posterior portion of each gland is filled with a granular material which appears to be derived from disintegrating cells filling the anterior part of the gland and lining its walls. A syncytium of transverse muscle fibers overlying these glands provides them with an effective means of contraction. Deep, slow, non-progressive contractions were observed repeatedly. The paired seminal vesicles are continuous with the anterior end of the ejaculatory duct and are packed with masses of quiescent sperm, the heads of which are directed against the opening of the ejaculatory duct. An unlined low epithelium with an overlying spiral layer

of muscle fibers comprises the wall of this organ. Lashing motions and non-progressive point contractions were visible in dissected seminal vesicles.

Changes occurring in the male copulatory apparatus during copulation. A profound change in the appearance of the male copulatory apparatus results when the flexor of the apodeme of the clasper contracts (Figs. 1–6). This movement

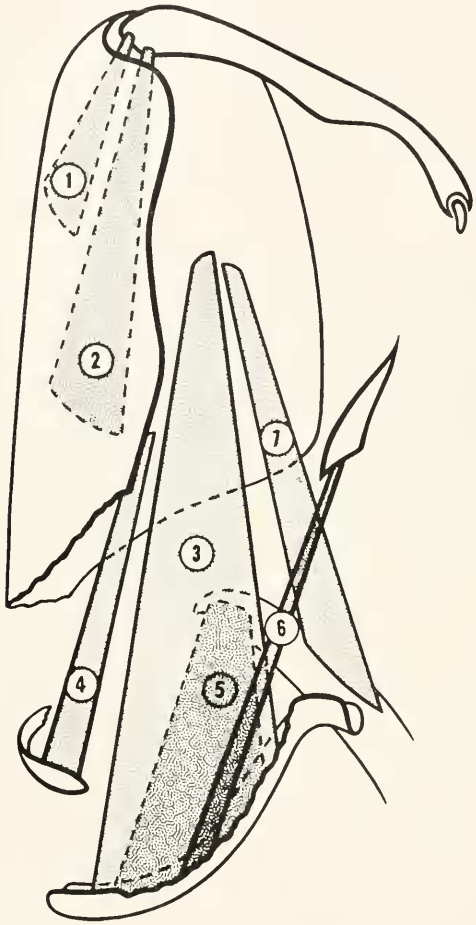
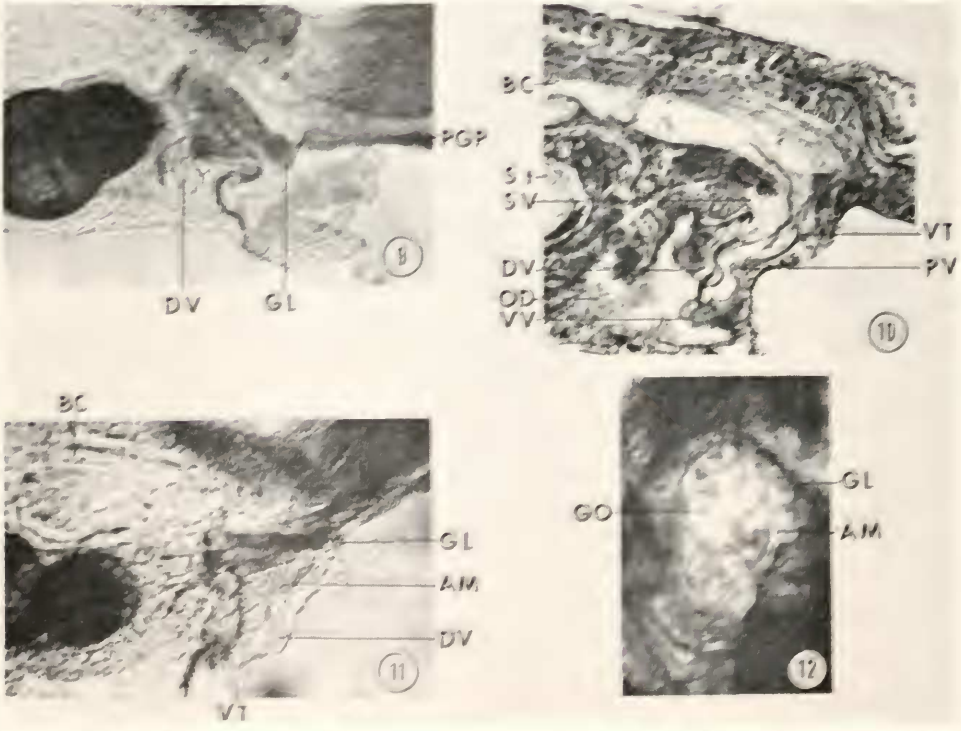


FIGURE 8. Diagram of the muscles of the male genital sclerites. 1, extensor of distal segment of clasper; 2, flexor of distal segment; 3, flexor of apodeme of clasper; 4, extensor of clasper; 5, flexor of apodeme of aedeagus; 6, flexor of lateral segment of paraproct; 7, extensor of apodeme of aedeagus.

initiates a chain of events that culminates in the erection of the aedeagus. The flexor rotates the ventral portion of the clasper apodeme dorsally and posteriorly and brings it in apposition to the body of the clasper. Thus, the apodeme, which is in a twisted position when the male is at rest, becomes straightened in copula and folded back toward the medial surface of the clasper. This process results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pore. In native, the erect aedeagus is roughly heart-shaped, and is armed with rows of strong teeth along its distal margin.

The eversion and extension of the aedeagus results in a radical change in the position of the paraphysis. The lateral cam-like projection, which is in close proximity to the aedeagus in the lyre by which the ends of the paraphysis is moved. The paraphysis hinges on its apodeme and extends by a counterclockwise direction due to pressure exerted against the cam by the aedeagus. The hook-like extension of the paraphysis is thereby projected beyond the lobes of the basal ring while the apodeme of the paraphysis remains normally stationary. The lateral segment is drawn toward the apodeme of the clasper by its flexor (muscle 8, Fig. 51), which serves to stretch the extended paraphysis and to draw the tool more rigidly against the ventral surface of the erect aedeagus.

Less radical changes occur in the position of other sclerites during copulation. The retraction of the distal lobe of the clasper draws that structure toward the basal plate of the clasper (muscle 2, Fig. 51). At the same time, the proximal lobe of the clasper is shifted dorsally by contractions of the muscles connecting them to the basal ring. The ventral lobes of the basal ring are connected to the apodemes of the three genital sclerites, and these structures tend to rotate ventrally.

Description of the female reproductive apparatus. The genital opening of the female is guarded by the genital lips, a circular membrane-like plate which is folded upon itself and articulates at this fold (Figs. 9–10). The lower lip bears an external extension, known as the tenda, while internally a pair of lobes, the genital rest, projects from the lip's dorsal edge. A transparent membrane, the atrial membrane, is continuous with the inner margin of the lips and includes a pair of sclerotized plates, the atrial plates (Figs. 15, 16). At rest, the genital lips are in contact with the upper lip extending somewhat beyond the lower one, and the atrial membrane unfolded.

The genital lips are continuous with the posterior, projecting post-genital plate, and are connected to the shell-like subgenital sternites. The post-genital plate, which separates the genital pit and openings, bears an external, elongated groove on its ventral surface. The tenda on the upper genital lip rests in correspondence channel with this groove. The external aspect of the groove serves as the insertion for the series of protractor muscles that overlap the commissure of the post-genital plate, tenda, and upper genital lip.

The internal organs of the copulatory apparatus of the female communicate with each other and with the genital opening through the complex, folded genital atrium (Fig. 14). The atrium has four openings: dorsally, it communicates with the copulatory bursa; ventrally with the common oviduct; anteriorly with the spermathecal ducts; and posteriorly with the genital lips. The duct from the oviduct that lines the upper portion of the atrium is ample. The S-shaped lower portion of the atrium is lined by a relatively heavy cuticle bearing many rows of denticle bases just below the junction of the spermathecal ducts. A layer of flat epithelial cells underlies the epithelial covering of the entire atrium. A series of three valve-like structures closes the lower atrium and appears to surround the opening of the common oviduct. The dorsal and ventral valves appear oval when cut from the atrium lumen in the lower plane. The posterior valve serves to close the lower atrium and, with the dorsal and ventral valves, guards the lumen of the oviduct.

The copulatory bursa is a blind sack that arises just internal to the upper genital lip. In virgin females, it is filled with an amorphous eosinophilic material and is composed of a thin layer of squamous epithelium with a light cuticular lining. The bursa extends anteriorly beyond the spermathecae where it is flattened dorsoventrally and folded upon itself. No contractions were seen in this structure during the course of the study and no muscles or nerves were found.

Female terminalia (corresponds to Figures 9–12).

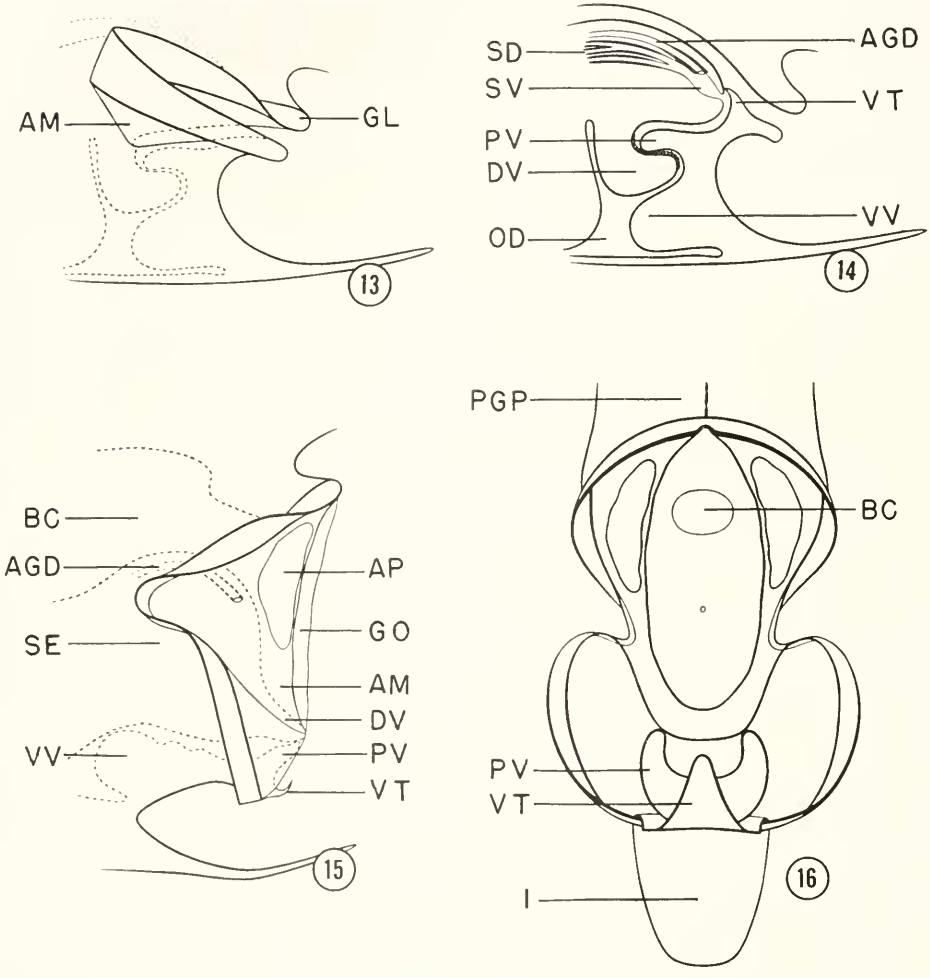

Figure 13. Lateral view of non-copulating female, showing infolding of atrial membrane (AM).

Figure 14. Sagittal section, showing structure of the atrium and spermathecal vestibule (SV).

Figure 15. Lateral aspect of copulating female, showing relationship of genital orifice (GO) to genital lips and posture of spermathecal eminence (SE) during coitus.

Figure 16. Caudal view, showing structure of extended atrial membrane.

The spermathecal eminence is a mound of fibrous connective tissue and transverse muscle fibers lying between the copulatory bursa and the common oviduct. A shallow, vertical groove marks its upper surface and terminates in a prominent cavity, the spermathecal vestibule (Figs. 10, 14). The accessory gland duct and the three spermathecal ducts communicate with the atrium through this cavity. The ducts of the two lateral spermathecae anastomose first. The duct of the medial spermatheca and the common duct of the lateral spermatheca fuse with the anterior wall of the vestibule, while the heavily cuticular accessory gland duct fuses with its mid-dorsal wall. The vestibule has no distinct lining and no muscles or valves are associated with its lumen. Its opening on the posterior-most surface of the spermathecal eminence appears to be guarded by the ventral tuft. Spasmodic twitching movements of the eminence are occasionally seen in fresh preparations and are apparently due to contractions of the transverse muscle bundle.

The three spherical spermathecae lie at the anterior end of segment VIII between the common oviduct and the copulatory bursa, and are partially embedded in a dense mass of visceral fat. The median spermatheca is the larger and the more anterior of the two lateral spermathecae. Each is a darkly pigmented, heavily cuticular, capsule which is naked on its inner, luminal surface. A series of minute pores is present near the orifice of each theca but not beyond the posterior third. Externally, a secretory cell is contiguous with each pore and surrounds a minute flask-shaped projection of the pore's rim. The nuclei and cytoplasmic granules of these cells are located in the portion of the cell away from the lumen of each spermatheca, and secretory vacuoles are sometimes present in the proximal portion of each cell. A thin squamous epithelium surrounds the remainder of the outer surface of the spermathecae. An extended constriction of the spermathecal capsule connects with the colorless layer of cuticle that lines the spermathecal ducts.

The spermathecal ducts are covered throughout their length by a thin epithelium. They emerge from the eminence anterior to the bifurcation of the duct of the smaller spermathecae. This anterior portion is covered by a layer of muscle tissue and an overlying membrane. Large cells, containing secretory vacuoles, are attached to the free portion of the ducts. They resemble the spermathecal gland cells, but are pedunculate and are attached to the spermathecal ducts by a narrow extension of cytoplasm. A fine, cuticle-lined capsule is present within each cell in an eosinophilic, non-granular, portion of the cytoplasm. Secretory vacuoles form in this region, but neither the capsule nor the vacuoles have been traced to the lumina of the ducts.

Description of the process of copulation. When abdominal contact is first established, the male's claspers engage the cerci of the female (Figs. 17–19). This brings the basal lobes of the claspers into contact with the female's post-genital plate. Specifically, the prominent hairs with recurved tips are directed into the groove on the ventral aspect of the post-genital plate. Following this contact, the aedeagus everts. Naturally-copulating pairs were observed repeatedly under conditions permitting recognition of the aedeagus if it were everted, but this was never seen unless the cerci were engaged. Experience with forced copulation confirms the observation that aedeagal eversion follows the establishment of abdominal contact.

As the aedeagus is everted, the apices of the paraprocts are extended and contact the lower genital lip of the female. Continued pressure by the aedeagus

through the paraprocts forces the lip ventrally. Thus, the opposing action of the basal lobes of the claspers and the paraprocts deforms the genital lips of the female. Eventually, the paraprocts come to rest with their cam-like projections

Photomicrographs of copulating pairs in lateral aspect.

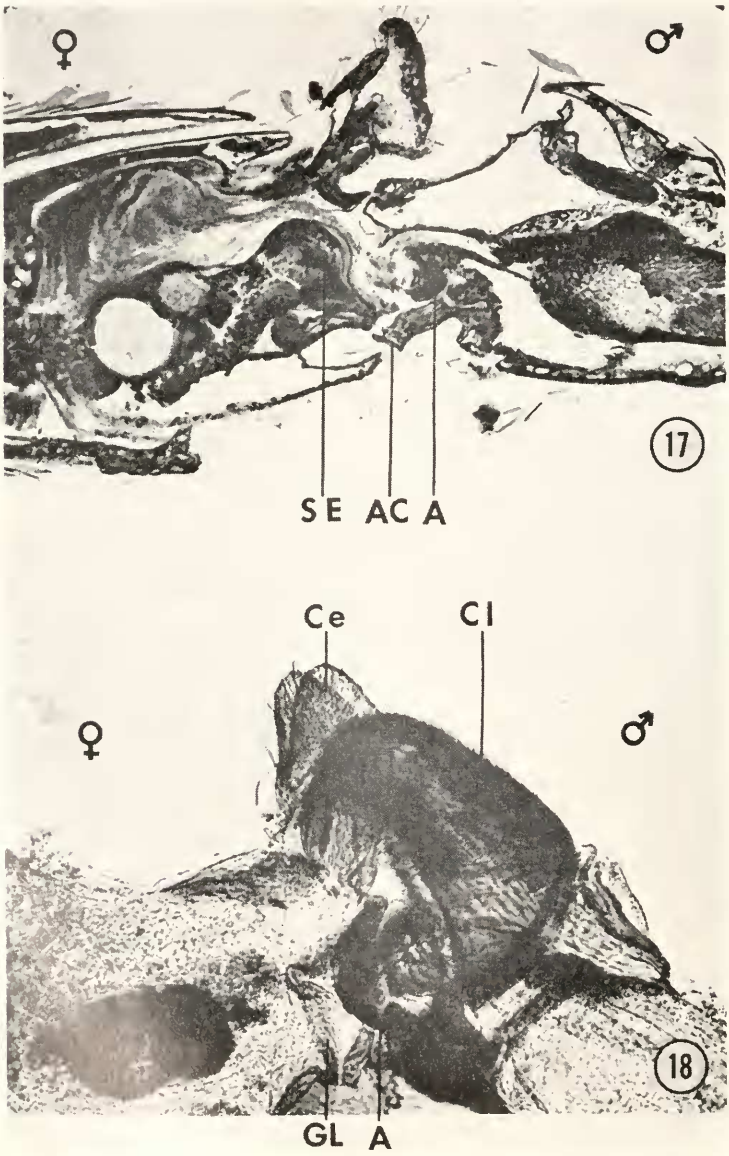

FIGURE 17. Sagittal section showing continuous channel formed by everted aedeagus and extended atrium of the female.

FIGURE 18. Whole-mount preparation, showing attachment of claspers to cerci of female and position of everted aedeagus relative to the genital lips.

placed in the folded hinge of the lips and with the hooked apices against the lower genital lip (Fig. 19).

The female's terminalia have now undergone a radical change in shape (Figs. 15, 16). The atrial membrane is everted when the genital lips are extended and the genital orifice is exposed. The orifice itself is at the end of this membranous, sleeve-like extension of the genital lips and precisely matches the outline of the everted aedeagus' dorsal extended surface. The most distal margin of the aedeagus is placed just inside the female genital orifice, but was never observed to be inserted even as far as the genital lips. The position of the aedeagus varied relative to the female, contact being established at any point along its outer margin.

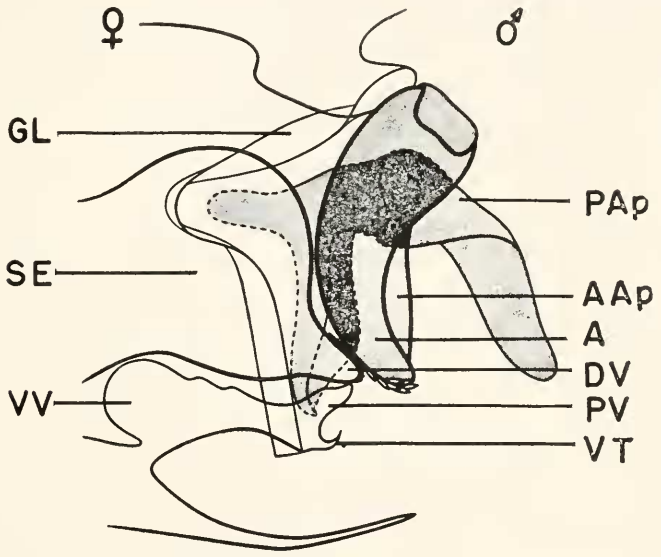

FIGURE 19. Position of aedeagus (A) and paraprocts (P) (both shaded) relative to the female during copulation (hemisected).

A firm seal is established between the extended genital parts of the copulating pair (Fig. 17). Laterally, the seal is formed by the spread lobes of the aedeagus within the atrial membrane. The paraprocts press against the female's atrial plates and in this manner serve to complete the lateral seal. Dorsally, the aedeagal pouch closes the space between the aedeagus and the upper genital lip, while ventrally, the seal is completed by the anal cone. The lateral lobes of the paraprocts stretch the anal cone against the aedeagus as the paraprocts are spread. The pair is thus joined in copula.

The shape of the female's atrium is disrupted as the atrial lips are distended. Most notably, the dorsal valve is drawn posteriorly and the posterior valve and ventral tuft are everted. The spermathecal eminence assumes a "goose head" appearance with the dorsal valve representing the beak. The spines on the dorsal surface of the valve are thereby exposed to the aedeagus of the copulating male. When in copula, the aedeagus lies against the outer surface of the dorsal valve

with its ventrally projecting spines engaging the dorsally projecting spines of the valve.

The mechanism of copulation may be represented by the following model. The male presents to the female the base of the cup-like aedeagus containing an ejaculatory apparatus in its vertex. The copulatory bursa of the female is extended to form an elongated blind pouch. These two structures are appressed, sealed, and subsequently inflated with seminal material. Deep intromission does not occur.

Transfer of sperm to the copulatory bursa of the female apparently results from contractions occurring in the male accessory glands and seminal vesicles. The ejaculatory duct is weakly muscled and appears to be a simple valve. This duct was fully distended in all ejaculating males sectioned. Deformations of the

TABLE II

Location of sperm in A. aegypti *females at intervals after termination of coitus (coitus interrupted after 10 seconds' duration)*

Time after coitus terminated	Number of females	Number of females with sperm in		
		Spermathecal vestibule	Spermathecae	Oviduct
25 sec.	1	0	0	0
30	3	0	0	0
35	2	1	0	0
40	2	2	1	0
45	2	1	1	0
50	2	2	0	0
55	2	2	0	0
60	2	2	2	0
2–5 min.	8	8	8	0
10	2	0	2	0
30	2	0	2	0*
60	2	0	2	2

* Sperm present in lower atrium in both females.

accessory glands and seminal vesicles, in addition, were observed solely in ejaculating males (Fig. 3). Sperm motility did not appear to play a role in transfer to the female. Sperm were invariably transferred in masses and were layered against the anterior wall of the bursa during copulation. It is suggested that the accessory glands provide the vehicle and the motive power through which the sperm are transported. Accessory gland contractions are strong and material is extruded from them when the integrity of the gland wall is disrupted (Lum, 1961). Contractions occurring in the seminal vesicles have less amplitude and it is suggested that their function is to release bundles of sperm into the streaming accessory gland fluid.

Observations on spermathecal filling. The fate of the seminal mass in the copulatory bursa was studied in females frozen at regular intervals after copulation. Some were sectioned (Table II) and others prepared as whole-mounts. In each freshly inseminated female studied, the copulatory bursa was inflated and the walls greatly distended. During coitus, sperm were deposited in four or five ven-

trally directed arcs intermixed with a granular gel which appeared to be derived from the male accessory glands (Fig. 17). Subsequently, sperm dispersed through the bursal contents.

The architecture of the atrium and of the atrial valves regained its precopulatory appearance within 30 seconds after termination of coitus. However, during the ensuing hour, the ventral tuft of each specimen continued to be separated from the spermathecal eminence by a space that exceeded the diameter of the vestibule. Subsequently (at about 35 seconds), sperm began to concentrate on the ventral wall of the bursa just above the spermathecal eminence. They were oriented toward the spermathecal vestibule with their heads directed into that structure. This mass of sperm, designated as the sperm plug, was present in each female in which spermathecal filling was observed (Table II). Among these females, sperm first appeared in the spermathecal ducts about 40 seconds after coitus and most females contained some sperm at 60 seconds. At this time, the sperm plug was very dense and contained a mass of cells which appeared to fill the lumen of the vestibule. In general, only a few sperm were seen at any one time in the ducts of sectioned material and these were invariably oriented with their heads toward the spermatheca. Although the accessory gland duct remained open, sperm never appeared to enter that structure.

At 5 minutes after coitus, sperm were scarce in the anterior portion of the copulatory bursa and the spermathecae appeared to be normally filled with sperm. Although the sperm plug was undiminished in size, sperm were rare in the spermathecal ducts. Simultaneously, the granular material contained in the bursa began to swell and to take on a "globular" appearance. This was accompanied by a thickening and vacuolization of the cells of the bursal wall.

The sperm plug was no longer present 10 minutes after copulation, and the remaining sperm appeared concentrated in the upper atrium. Small numbers of sperm were recognized in the lower atrium beneath the level of the spermathecal vestibule at 30 minutes.

At one hour after coitus, the common oviduct contained masses of agglutinated, immobilized sperm. The bursa was filled with coarse material and the remaining sperm were compressed into its posterior end and into the upper atrium. The bursal wall was greatly thickened and much vacuolated and the bursa itself reduced in size. By the next day, the globules had been replaced by amorphous, granular material, and the bursal walls had become squamous. Although motile at this time, bursal sperm lost their motility by the second day after coitus.

Discussion

The aedeagus does not function as a deep intromittent organ in *Aedes aegypti*. Rather, seminal material is transferred to the female through close apposition of genital parts of the copulating pairs. This precise mechanical relationship between the sexes during copulation recalls Dufour's "lock and key" hypothesis. *A. aegypti* and *A. albopictus* are mechanically incompatible (Leahy, 1962), and semen wasted in such matings has been found externally on the abdomens of females. Thus, terminalia of each member of a mosquito population must be of constant form and dimension, a phenomenon that has been exploited by taxonomists for many years.

Intromission occurs during copulation in various Diptera. Male *Phlebotomus* spp. possess a tube-like genital filament that is inserted into the spermathecal ducts of the female (Hertig, 1949). Sperm are thus deposted directly into the spermathecae in these organisms. Anophelines (but not culicines) have a similar filament that lies within the folds of the aedeagus (Hodapp and Jones, 1961). The aedeagus of the copulating *Anopheles stephensi* illustrated by Russell and Mohan (1939) appears to be placed superficially upon the body of the female. Comparable slide material supplied by B. N. Mohan confirmed this impression. Taken together, this suggests that anophelines may copulate in a manner analogous to that of *Phlebotomus*, that is, through deep intromission of a genital filament. Giglioli (1963), however, theorized that sperm of *A. gambiae* are discharged near the orifice of the spermathecal duct and that they subsequently migrate to the spermathecae. Snodgrass (1963) emphasized that the aedeagus need not be regarded as an organ of deep intromission and the present study supports this concept.

In contrast, *A. aegypti* sperm are delivered to the copulatory bursa, and do not transfer to the spermathecae until after the copulating pair has separated. Transfer commences at approximately 40 seconds after copulation and continues for several minutes. Those sperm that fail to transfer to the spermathecae, together with the seminal fluid, are subsequently digested and absorbed in the bursa.

The correct location of the genital openings in *A. aegypti* has not previously been described. It has generally been assumed that the male genital opening is located at the apex of the aedeagus (Hodapp and Jones, 1961; Christophers, 1960), and the female opening was thought to be bordered by the genital lips (Christophers, 1960). The aperture located above the upper genital lip of an *Aedes aegypti* female described and figured by Curtin and Jones (1961) is perhaps an artifact. This study demonstrates that the male genital opening is located at the base of the aedeagus and the female opening at the apex of the everted atrial membrane. Atrial plates were recognized by Coher (1948) in species of a number of culicine genera but not in *Aedes*. However, our observations, as well as those of Burcham (1957), demonstrate that they also occur in *A. aegypti*. The function of these plates must be that of supporting the atrial membrane, and of extending the female genital opening.

While these descriptions of the anatomy of the internal components of the male terminalia are in general agreement with those of Christophers (1960) and of Hodapp and Jones (1961), our observations upon the female differ in several respects from published reports. The arrangement of the atrial chambers and the structure of the atrial membrane have not been previously reported. However, the ventral tuft (vaginal membrane or vaginal valve) was noted by Parks (1955) and Burcham (1957). Leahy (1962) suggested that this tuft was a filtering organ which separated sperm from accessory gland material after termination of copulation. However, the function of the spermathecal eminence and the relationships between the spermathecal gland ducts, the accessory gland ducts and the atrium have remained obscure. Christophers (1960), and Curtin and Jones (1961) did not describe this region in detail. The presence of the thin-walled spermathecal vestibule connecting the ducts with the atrium is described for the first time. The dorsal plate, described by Curtin and Jones (1961), was not observed and probably represents the displaced genital lips of the female.

The peculiar ducted glands surrounding the base of the spermathecae and occurring in clusters along the spermathecal ducts are analogous in structure to the cells of the female accessory gland. Each cell contains a minute, flask-shaped, cuticular duct within its cytoplasm. Vacuoles, presumably secretory in nature, are contiguous with the ends of these ducts, and the nuclei and granular elements of the cell's cytoplasm are displaced to the opposite end of the cell. It is suggested, therefore, that each of these cell types is secretory. The spermathecal glands secrete into the spermathecae through the ducted pores around their base. The glands surrounding the spermathecal ducts appear to secrete into the ducts through their own minute ducts which presumably pierce the substance of the spermathecal ducts. The spermathecal ducts of *Anopheles gambiae* are pierced by the ducts of similar cells (Giglioli, 1963). Similarly, the female accessory gland apparently secretes directly into the spermathecal vestibule. Although the functions of these glands are unknown, they may be presumed to have some role in insemination.

The sensory stimuli that control copulation are apparently multiple. The sound of the female's wings (Roth, 1948), indeed, attracts males and induces them to perform certain pre-copulatory behavior patterns. Once the male has been primed by the sound of her wings, tarsal contact appears to induce further copulatory behavior. However, aedeagal eversion appears to be stimulated exclusively through contact of the male's terminalia with those of the female. It is suggested that the prominent, recurved sensillae on the basal segment of the clasper may receive such a stimulus from the post-genital plate of the female. Rees and Onishi (1951) observed that mechanical stimulation of these hairs caused movement in the aedeagus of *Culiseta inornata* males interrupted while copulating. Copulation of restrained *Aedes* pairs can be induced by placing the terminalia in contact (McDaniel and Horsfall, 1957). Thus, various stimuli mediated through the terminalia, as well as through the tarsi and antennae, control copulation and sperm transfer.

Summary

1. The aedeagus of the *Aedes aegypti* male is placed superficially within the genital orifice of the female during copulation and is not a deep intromittent organ. When everted, it rotates through more than 90 degrees and its lobes spread, revealing the genital pore in its base. The paraprocts' function is to distend the genital lips of the female and to elevate the anal cone of the male. During coitus, the sleeve-like atrial membrane of the female is everted, revealing the genital opening at its apex. Sexual union is accomplishd through the junction of the everted aedeagus and the atrial membrane. The anal cone, the aedeagal pouch and the paraprocts assist in the formation of a firm line of union. The mechanics of eversion of the aedeagus and atrial membrane and the details of their juxtaposition are analyzed.

2. It is suggested that contractions of the male accessory glands provide the current in which the sperm are carried to the copulatory bursa of the female, and that the sperm subsequently transfer to the spermathecae.

3. The anatomy of the genital atrium of the *A. aegypti* female is described. The valves of the lower atrium seal the oviduct of the non-ovipositing female. The dorsal valve is everted during copulation and its spinous outer surface engages the

spines of the aedeagus. The spermathecal ducts and accessory gland duct communicate with the atrium through a common chamber.

4. Males receive complex mating stimuli from the female and require auditory as well as other kinds of stimuli before sperm transfer is accomplished.

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